FREEING CHRYSANTHEMUMS FROM THE ROD-SHAPED LEAF MOTTLING VIRUSES AND TOMATO ASPERMY VIRUS BY A COMBINATION OF MERISTEM-TIP CULTURE WITH HEAT-TREATMENT

Masayoshi ASATANI

INTRODUCTION

There is abundant evidence that virus diseases are widespread in Japanese chrysanthemum stocks. Among them affections with tomato aspermy virus (TAV) and rod-shaped leaf mottling virus group typified by virus B(CVB) are the most prevalent, and followed by ring patterns, the incitant of which is at present regarded as a virus though little is known about its entity. The presence of TAV and the rod-shaped leaf mottling viruses (hereafter abbreviated as leaf mottling viruses) in Japanese chrysanthemums and other composite plant has not been confirmed until recently (2, 18, 30, 31). It is suggested that TAV plays a part in the deterioration of flowers and debilitation of plants, but as to its own role the results so far reported do not coincide with each other (4, 11, 23). CVB, on the other hand, induces in graft-inoculated plants of many cultivars only a transient leaf mottle, which fades out in a few weeks, and plants with long-standing infections are usually symptomless (4, 12). One of the leaf mottling viruses, however, produces in some cultivars stable vein mottle which persists throughout the all seasons (3). Also, brown streaking of ray floret, smaller flowers, later blooming time, shorter stalks and poor lasting quality are ascribed to CVB (5, 11). In several chrysanthemum-growing districts many valuable cultivars have been wholly infected with viruses. But it is usually difficult for growers to obtain great numbers of healthy cuttings at a time, for specialized producers of healthy cuttings are not present.

Nowadays two methods are known to be practicable to obtain virus-free chrysanthemums: one is heat-treatment and the other is meristem-tip culture. After 3–4 weeks' incubation at 36°C, Hollings and Kassanis cured chrysanthemums from tomato aspermy, stunt (English strain) and ring pattern viruses, but not from viruses B, D, vein mottle virus and an unidentified virus (13). Larsen failed to cure chrysanthemums from infection with stunt virus (American strain) after 12 weeks' heat-treatment at 38°C (22). Brierley and Smith could obtain two cultivars of chrysanthemum free from flower distortion (its etiology may need to be reexamined because the yellows type diseases of plants induced by mycoplasma-like organisms have recently been increasing) after more than 2 months at 35°C (6). Brierley and Lorentz treated TAV- and

mosaic-infected chrysanthemums at 35°C up to 10 months and obtained cuttings free from both viruses (5). Stone and Hollings, however, could not effectively eliminate stunt virus from mini-cuttings taken after 9 months' treatment at 35°C, while in combination with meristem-tip culture they could readily obtain stunt-free plants (29). Hakkaart and Quak also, using a combination of both methods, eradicated CVB from chrysanthemums in higher proportions than those attained by meristem-tip culture alone (9). In Japan Kimura (21) and Mori *et al.* (24) tried to culture meristem-tips to resuscitate several chrysanthemum cultivars from apparent virus infections, although they made only visual inspection for the virus indexing before and after culturing. In this study, of the viruses in chrysanthemums, only two types of leaf mottling viruses and TAV were investigated on their behavior in chrysanthemum plants in response to high temperature and subsequent culture of meristem-tips.

MATERIALS AND METHODS

Plants of the 4 cultivars, "Hakuho", "Bansei-Alps", "Unaruga" and "Fuku-un", were all proved to be infected with the leaf mottling viruses. Of these viruses three were CVB and one in "Unaruga" plants appeared to be vien mottle virus described by Hollings (12); this is discussed later. Plants of "Fuku-un" were infected also with TAV. No more symptoms were observed in plants of "Hakuho" and "Bansei-Alps", whereas clear ring and line patterns typical of the ring patterns disease persisted for a short period after they had been collected. There were also no symptoms in "Fuku-un" plants that had shown transient ringspots distinct from above-mentioned ring patterns just after the collection of them. "Unaruga" plants, on the other hand, exhibited vein mottle, partial vein necrosis, severe distortion and crinkling of leaves throughout the year.

Rooted cuttings from each of the cultivars were transplanted into wooden boxes, 15 cm deep, 20 cm wide and 40 cm long, the number of plants being 20 per box. When the plants in the boxes began to grow vigorously, they were topped and in further 2 weeks the boxes were placed in a heat incubator. The incubator was a miniature glasshouse, whose ridge roof was cut horizontally just under the central ridge, thus having two parallel ridges and a narrow flat roof at the center. It was made of wooden frame 100 cm high, 90 cm wide and 180 cm long; it was walled and roofed with vinyl sheet 0.2 mm thick, upon which were punched 32 holes, 0.6 cm in diameter, in 4 rows along the central ridges on the flat portion of the roof, and opened to the outside from one of the long side. It had a slatted wooden floor raised 15 cm from the bottom, and 3 steel pipe heaters (straight fin type), each 0.5 kilowatts, mounted on a frame of angle iron, were fixed at 5 cm from the bottom, and narrow asbestos

baffle boards separated the heaters from the floor. It was thermostatically controlled and a self-regulating thermometer was placed in it. A small fan (Type MFQ, Oriental Motors Co., Ltd.) to circulate the air was suspended from the ridges and set to turn only when the heaters were working. The incubator was placed on a glasshouse bench, and adjusted to keep the air temperature at 38±1°C, but this temperature was often exceeded at maximum by about 5°C for 2-3 hours around midday though a double curtain of cheesecloth was drawn to intercept the sunshine. To prevent initiation of flower buds, plants were kept in vegetative growth, being submitted to supplementary illumination for 3-4 hours in the night. Plants in each box were treated for 1, 2, 4, 6 and 8 weeks prior to excision of meristem-tips. Control meristem-tips were also excised from the plants that had not been heat-treated. Treatments were continued from November 1968 to July 1969.

Two liquid media for aseptic culture were prepared: medium of Murashige and Skoog (MS medium) (25) containing 0.2 mg kinetin and 2 mg naphthalene actic acid (NAA) in place of indole-3-acetic acid per liter, and Neergaard's medium free from NAA (N medium) (28). Meristem-tips were excised from shoot apices or axillary buds under dissecting stereomicroscope with a fragment of razor blade held in a razor blade forceps. They were excised as tissue blocks with 4 vertical and one horizontal sections, and had sizes ranging from 0.3 mm to 0.5 mm in height, usually including two leaf primordia. The tissue, without surface-sterilization, was first attached to the wet tip of a hooked platinum wire 0.5 mm in diameter that was used as a standard for the determination of the size of tissue unit, and then transferred onto a filter paper bridge stood in test tube, 1.3 cm in inside diameter and 10.5 cm long, containing 2.5 m*l* of MS medium. Test tubes were capped with aluminum foil and incubated at 20°C (at higher temperatures in warmer seasons). When root and/or shoot initials were visible, or dedifferentiation of the tissue overwhelmed any sign of redifferentiation, the medium was poured off and N medium was poured into the test tube. When roots and shoot had elongated enough, usually at later than the 3 leaf stage, the plantlets were shifted to small pots 4.5 cm in diameter containing autoclaved vermiculite, placed on glasshouse benches under a wooden frame overlaid with cheesecloth, and sprayed periodically with ½,000 dilution of Hyponex. When plants began to grow with vigor, they were then transferred into pots 9 cm in diameter containing autoclaved soil, and grown in groups on glasshouse benches, each being enclosed witn cheesecloth canopy 50 cm high and 90 cm wide and long to exclude aphid attack. At least in 6 months after the plantlets had been taken out of the test tubes, a few younger leaves were detached from the explants, ground in mortar,

and sap-inoculated on to the leaves of clonal cuttings of *Petunia hybrida*, one sample per plant, for indexing of the leaf mottling viruses and TAV. But when TAV was present, it interfered with indexing of the leaf mottling viruses, inducing earlier appearance of local lesions similar to those produced by the latter viruses on the petunia leaves inoculated. The explants were then tested again for the presence of the rod-shaped viruses electron microscopically.

RESULTS

From 23 lots of plants of the 4 cultivars a total of 1377 meristem-tips were cultured, of which 764 or 55.5% survived to become plants. The proportions of the survived plants of the 4 cultivars, i. e., "Hakuho", "Bansei-Alps", "Unaruga" and "Fuku-un", were 81.2%, 51.5%, 26.7% and 55.4%, respectively (Table 1). Plants of the cultivar "Hakuho" were not only high in the proportion of survivors, but also fast in initiation and subsequent growth of roots and shoots, and MS medium was substituted for N medium at least in 14 days. In general, "Fuku-un" comes after "Hakuho" both in the proportion of survivors and the rate of growth, although high temperature in the summer caused drying and microbial attacks, killing numbers of plantlets of this cultivar just before and after transfer, and resulted in the decrease of the proportion of survivors. As greenish callus grew predominantly from the meristem-tips of the plants of "Bansei-Alps", MS medium was often exchanged for N medium before the initials of plant organs were visible. The resulting dilution of the traces of remaining NAA and kinetin with the new N medium stimulated the callus to redifferentiate into plant organs. A small number of plants, however, had become large without roots in the test tubes, and mini-cuttings taken from them were then planted into small pots containing autoclaved vermiculite. Most meristem-tips of "Unaruga" plants induced callus growth with as much vigor as observed in "Bansei-Alps", while the medium exchange did not so effectively stimulate the redifferentiation of callus as did in the case of "Bansei-Alps". Many "Unaruga" meristem-tips increased in their sizes, producing only brown callus irrespective of repeated exchange of the medium, but sudden growth of shoots and roots succeeded sometimes in several months. In general, survival of the excised meristem-tips of all the cultivars did not seem to be influenced by the length of heat-treatment. Furthermore, of the 368 meristem-tips excised from "Fuku-un" plants, 266 were taken from shoot apices and 102 were from axillary buds, and a comparison was made of the proportions of survivors among the lots of treatment. I could not, however, recognize any survival superiority of the meristem-tips excised from shoot apices over those from axillary buds.

TABLE 1

Effect of length of heat-treatment on freeing chrysanthemum plants from virus B (CVB), vein mottle (VMV) and tomato aspermy (TAV) viruses by means of meristem-tip culture*

Length of heat-treatment (weeks)	No. of meristem-tips excised	No. of plants survived	No. of plants free from CVB or VMV	No. of plants free from TAV		% of plants survived	% of plants free from CVB or VMV	% of plants free from TAV
			"Hakuho"					
None	39	36	36			92.3	100.0	
1	43	42	40			97.7	95.2	
2	63	50	48			79.4	96.0	
4	90	48	46			53.3	95.8	
6	61	57	56			93.4	98.2	
8	50	48	48			96.0	100.0	
Totals	346	281	274		Aver.	81.2	97.5	
			"Bansei-Alps"					
None	48	22	22			45.8	100.0	
1	41	29	29			70.7	100.0	
2	61	39	39			63.9	100.0	
4	149	65	65			43.6	100.0	
6	78	51	51			65.4	100.0	
8	35	6	6			17.1	100.0	
Totals	412	212	212		Aver.	51.5	100.0	
			"Unaruga"					
None	51	11	11			21.6	100.0	
1	—	—	—			—	—	
2	49	1	1			2.0	100.0	
4	60	16	16			26.7	100.0	
6	50	17	17			34.0	100.0	
8	41	22	22			53.7	100.0	
Totals	251	67	67		Aver.	26.7	100.0	
			"Fuku-un"					
None	103	63	62	61		61.2	98.4	96.8
1	61	31	31	31		50.8	100.0	100.0
2	23	18	18	18		78.3	100.0	100.0
4	50	28	28	28		56.0	100.0	100.0
6	80	32	32	32		40.0	100.0	100.0
8	51	32	32	32		62.7	100.0	100.0
Totals	368	204	203	202	Aver.	55.4	99.5	99.0

* Meristem-tips, 0.3-0.5 mm long, were aseptically cultured first in test tubes containing liquid medium of Murashige-Skoog (MS medium) [0.2mg kinetin and 2.0mg NAA in place of IAA per liter], which was then substituted for Neergaard's medium without NAA (N medium). "Unaruga" plants were infected with VMV, and plants of the other cultivars were with CVB. "Fuku-un" plants were infected also with TAV.

As shown in table 1, the explants derived from all the lots of plants of the 3 cultivars, i. e., "Bansei-Alps", "Unaruga" and "Fuku-un", that had been heat-treated for more than one week were all free from the leaf motting viruses. On the other hand, CVB survived in 4.8%, 4.0%, 4.2% and 1.8% of the "Hakuho" explants obtained from the lots of plants that had been pretreated with heat for 1, 2, 4 and 6 weeks, respectively. In contrast, the control explants derived from "Hakuho" as well as "Bansei-Alps" and "Unaruga" plants were proved to be all free from the leaf mottling viruses except for the one from a "Fuku-un" plant. On the other hand, TAV was eradicated from all the lots of plants that had been heat-treated for longer periods than one week, while 3.2% of the explants in the control lot were not TAV-free. It is of great interest that two "Fuku-un" explants which are not cured from infection with TAV are free from CVB, and that one still affected with CVB is free from TAV.

DISCUSSION

Although we have formerly designated tentatively the virus associated with the plants of the cultivar "Unaruga" as CVB (1, 3), it seems likely that vein mottle virus of Hollings (12) is identical with, or similar to, the virus under the present study, for the two viruses have some features in common. Severe vein mottle is the typical symptom of the vein mottle virus and its titer in chrysanthemums in the summer is higher than that of CVB (12), while constant vein mottle, accompanying partial vein necrosis and deep marginal crinkling at higher temperatures, is also characteristic of the virus present in "Unaruga" plants. Milder symptoms plus epinasty also were produced in the plants of another cultivar "Amagahara" when scions of "Unaruga" plants were grafted on to them. Stability of symptoms and higher virus titer in the summer were not characteristic of the isolates of CVB used in this study, whose symptoms were in general transient and faded out sooner or later. On the other hand, "Hakuho" and "Bansei-Alps" plants exhibited clear ring and line patterns on leaves from autumn to the next spring after they had been collected, but thenceforth the symptoms never appeared. The symptomatic transientness and the lability of the etiological entity to higher temperature are also resemble the properties of the ring pattern virus of Hollings and Kassanis (13). Therefore, it is considered probable that "Hakuho" and "Bansei-Alps" were infected with both CVB and ring pattern virus, "Unaruga" was with vein mottle virus, and "Fuku-un" was with TAV, CVB and an unidentified virus which also produced temporary ringspots only for a short period after collection. The classification of the rod-shaped viruses in chrysanthemums, however, affords scope for discussions, and should be rearranged in the future on the

basis of the grafting method including the application of the selected numbers of chrysanthemum cultivars. But ultimately, the classification of these viruses will entirely depend on serology.

According to Kassanis and Posnette all the rod-shaped viruses tested by them multiplied at 36°C (20). Hollings and Kassanis could not eliminate rod-shaped viruses such as CVB, chrysanthemum virus D and vein mottle virus from chrysanthemum plants by keeping them at 36°C for 3-4 weeks (13). Of the eight mosaics of chrysanthemums reported by Brierley and Smith (7), at least six may be included in the leaf mottling virus group under discussion solely because of their reactions in petunia. Among them the incitants in the plants of cultivars "Superlative" and "Blanche" were found especially tolerant of heat, no healthy scions being reclaimed after 6 months' treatment at 35°C (7). Brierley and Lorentz also obtained CVB-free cuttings from heat-treated chrysanthemums imported from Japan and Taiwan after 2-10 months at 35°C (5). Similarly, in our earlier experiment, in which only vein mottle virus (previously referred to as CVB) was tested, virus-free explants were recovered from the cuttings taken from shoot tips of "Unaruga" plants that had been kept at 38°C for 4 weeks (3). This indicates that heat-treatment alone can be an effective method to obtain plants free from the leaf mottling viruses if the smallest cuttings are taken from the treated plants. According to Hakkaart and Quak keeping plants at 37°C for 10 to 60 days before excising meristem-tips affected markedly the proportions of CVB-free explants (9). For example, only 9 to 20% of explants free from CVB were obtained singly by meristem-tip culture, while more than 90% of the explants could be freed from CVB when the parent plants had been pretreated with heat (9). However, CVB was not completely eliminated from any batch of the explants derived from the heat-treated parent plants except one, in which all of the 6 explants were CVB-free (9). But this figure is too small to be appraised statistically. Hollings and Stone also seem to have attained the high proportion of healthy chrysanthemums recovered from the leaf mottling viruses by means of meristem-tip culture and heat-treatment (14). In the present study, however, 100% of explants were freed from the leaf mottling viruses with three cultivars and 98.4% with one cultivar only by means of meristem-tip culture. Eventually, there seems to be no correlation between the lengths of heat-treatment and proportions of explants free from the leaf mottling viruses. Above all, the prolongation of heat-treatment had little effect on the increase in the proportions of CVB-free explants from "Hakuho". The fact that CVB in "Hakuho" plants alone was tolerant of heat up to 6 weeks can not be explained by the errors in excising proper sizes of meristem-tips, for the meristematic domes of

" Hakuho " plants are not so large as to exceed 0.5 mm in height whether plants are heat-treated or not. Furthermore, with potato viruses X and S, it is known that, as heat-treatment progresses, larger buds of potatos are likely to be virus-free (27). Therefore, this finding may reflect the heat tolerance of the virus itself in " Hakuho " plants. The concentration gradient of the virus toward the shoot apex may not be so affected as to decrease instantly by exposure to heat. As described, the leaf mottling viruses were completely eradicated from all the lots of explants from the cultivars other than "Hakuho", i.e., "Bansei-Alps", "Unaruga" and "Fuku-un", that had been pretreated with heat. But it was also demonstrated that pretreatment of plants with heat was not essential for elimination of these viruses. Thus, without heat-treatment high proportions of explants free from the leaf mottling viruses can be attained only by meristem-tip culture whenever meristem-tips shorter than 0.5 mm are excised.

Holmes obtained chrysanthemums free from TAV only by grafting TAV-infected scions on to healthy plants, but CVB could not be eliminated by this method (17). This grafting method, however, is too low in its efficiency to be practicable today. Kassanis obtained for the first time shoot cuttings as well as parent plants free from TAV, after keeping tobacco and tomato plants affected with TAV at 36°C for 3 weeks or more (19). Hollings and Kassanis (13) and Brierley and Lorentz (5) eliminated TAV from chrysanthemums by heat-treatment alone. We have already reported that the parent plants of " Unaruga " themselves were permanently freed from TAV after heat-treatment, while symptoms of vein mottle reappeared soon after they were returned to the temperatures in the glasshouse (3). Thus, TAV is labile to heat, and eradicated far more readily from chrysanthemums only by excising small cuttings from heat-treated plants than by laborious culturing of meristem-tips. As described, 61 of the 63 explants derived from meristem-tips of " Fuku-un " plants in the control lot were proved to be TAV-free, whereas 2 TAV-infected explants were free from CVB. On the other hand, the sole explant in the same lot that was not cured from infection with CVB was shown to be free from TAV. No explant obtained from " Fuku-un " plants has so far been proved to be infected with both viruses at the same time. However, Stace-Smith and Mellor reported that, when the meristem-tips with larger sizes were dissected and cultured, potato explants were not in some cases freed from even one of the two rod-shaped viruses, potato viruses X and S (27).

Since " Bansei-Alps " and " Hakuho " plants had already been symptomless before heat-treatment and aseptic culture began, I had no means of indexing for ring pattern virus, and could not confirm the results of Hollings and Kassanis that ring pattern virus was labile to heat (13).

In the present study, I propagated 4 isolates of the leaf mottling viruses in the corresponding cultivars and an isolate of TAV in a cultivar. Nevertheless, for the precise comparison of the behavior among the 4 isolates of the leaf mottling viruses, all of the isolates should be multiplied in the plants of the same cultivar, e. g., "Hakuho" from which the highest proportions of explants are expected to be available, because it is still inconclusive whether there may be any correlation between the following two facts: only "Hakuho" explants could not be freed from the virus among all lots of explants of the 4 cultivars whose parent plants had been pretreated with heat; the explants derived from the meristem-tips of "Hakuho" survived in the highest proportions among all the explants tested. Critical sizes of meristem-tips to be excised also should be determined with each combination of cultivars and virus isolates. Finally, it is possible that indexing of the leaf mottling viruses only by inoculation test on petunia plant is not sensitive enough particularly when the virus titer in chrysanthemum plants drops as experienced in the summer. Therefore, while attempts to find out more sensitive test plants have been continued, electron microscopy and serology have been introduced for the indexing of this type of viruses (8). At present, electron microscopy is the most sensitive and quickest method, though its application to indexing is limited by the difficulty in determining which virus of this virus group is present (14). For this reason, serological methods should first of all be established when high-titered antisera to this virus group become available. And this area of investigation has already been being fertilized by the efforts of several working groups (10, 14, 15, 16, 26).

Acknowledgement I am indebted to Prof. U. Hiura and Asst. Prof. T. Inouye for their incessant encouragement during this study, and to Mr. T. Fukuyama for his valuable discussions on the results. Thanks are also expressed to Prof. K. Kano, Kagawa Univ., and to Mr. K. Kimura, Kagawa Agric. Exp. Sta., for their kind instructions in the techniques of meristem-tip culture. The excellent technical assistance of Mr. K. Mitsuhata is gratefully acknowledged.

SUMMAY

Cultivars of chrysanthemums, including "Hakuho" and "Bansei-Alps" affected with virus B (CVB), "Unaruga" with vein mottle virus and "Fuku-un" with CVB and tomato aspermy virus (TAV), were heat-treated at 38°C for 1, 2, 4, 6 and 8 weeks. Of the 1377 meristem-tips taken from all the lots of parent plants pretreated with or without heat, 764 explants survived. In the case of "Hakuho" plants, 95.2%, 96.0%,

95.8% and 98.2% of explants in the 1, 2, 4 and 6 weeks' lots, respectively, were freed from CVB, while in the control and 8 weeks' lots 100% of healthy explants were reclaimed. In contrast, in the cases of "Bansei-Alps", "Unaruga" and "Fuku-un" plants, all the explants derived from all the lots of plants except the control lot of "Fuku-un", in which still 1 of the 63 explants was infected with CVB, were eradicated from CVB or vein mottle virus. On the other hand, TAV was completely eliminated from all the lots of "Fuku-un" explants whose parent plants had been heat-treated, while 2 of the 63 explants in the control lot were still affected with TAV.

LITERATURE CITED

1. Asatani, M. 1970. Freeing chrysanthemums infected with chrysanthemum virus B and tomato aspermy virus by means of heat-treatment and meristem-tip culture. Ann. Phytopath. Soc. Japan 36: 356-357 (Abstr.).
2. Asatani, M. 1970. Viruses isolated from *Aster savatieri* Makino. Ann. Phytopath. Soc. Japan 36: 185 (Abstr.).
3. Asatani M., and Inouye, T. 1968. Thermotherapy of virus-infected chrysanthemums (Preliminary report). Ann. Phytopath. Soc. Japan 34: 384-385 (Abstr.).
4. Brierley, P. 1955. Symptoms induced in chrysanthemums on inoculation with the viruses of mosaics, aspermy, and flower distortion. Phytopathology 45: 2-7.
5. Brierley, P., and Lorentz, P. 1960. Healthy tip cuttings from some mosaic-diseased Asiatic chrysanthemums: some benefits and other effects of heat treatment. Phytopathology 50: 404-408.
6. Brierley, P., and Smith, F. F. 1957. Symptoms of chrysanthemum flower distortion, dodder transmission of the virus, and heat cure of infected plants. Phytopathology 47: 448-450.
7. Brierley, P., and Smith, F. F. 1958. Some characteristics of eight mosaic and two rosette viruses of chrysanthemum. Plant Dis. Reptr. 42: 752-763.
8. Hakkaart, F. A. 1969. A comparison of electron microscopy and serology with infectivity tests for the detection of chrysanthemum virus B. Neth. J. Plant Path. 75: 355-359.
9. Hakkaart, F. A., and Quak, F. 1964. Effect of heat treatment of young plants on freeing chrysanthemums from virus B by means of meristem culture. Neth. J. Plant Path. 70: 154-157.
10. Hakkaart, F. A., van Slogteren, D. H. M., and de Vos, N. P. 1962. Chrysanthemum virus B, its serological diagnosis in chrysanthemum, and its relationship to the potato viruses S and M and carnation latent virus. Tijdschr. PlZiekt. 63: 126-135.
11. Hollings, M. 1955. Investigation of chrysanthemum viruses. I. Aspermy flower distortion. Ann. appl. Biol. 43: 86-102.
12. Hollings, M. 1957. Investigation of chrysanthemum viruses. II. Virus B (mild mosaic) and chrysanthemum latent virus. Ann. appl. Biol. 45: 589-602.
13. Hollings, M., and Kassanis, B. 1957. The cure of chrysanthemums from some viruses by heat. J. Roy. Hort. Soc. 82: 339-342.
14. Hollings, M., and Stone, O. M. 1967. Rept. Glasshouse Crops Res. Inst. 1966 (a typewritten copy sent from Hollings).
15. Hollings, M., Thorne, J. M., and Atkey, P. T. 1970. Rept. Glasshouse Crops Res. Inst. 1969: 128-129.
16. Hollings, M., Stone, O. M., and Thorne, J. M. 1971. Rept. Glasshouse Crops Res. Inst. 1970: 149-150.

17. Holmes, F. O. 1956. Elimination of aspermy virus from the Nightingale chrysanthemum. Phytopathology 46: 599-600.
18. Inouye, T., Astani, M., and Mitsuhata, K. 1968. Studies on the viruses of plants in *Compositae* in Japan. I. Tomato aspermy virus from chrysanthemum. Nogaku Kenkyu 52: 55-64 (in Japanese).
19. Kassanis, B. 1954. Heat-therapy of virus-infected plants. Ann. appl. Biol. 41: 470-474.
20. Kassanis, B., and Posnette, A. F. 1961. Thermotherapy of virus-infected plants. Recent Advances in Botany 1: 557-563.
21. Kimura, K. 1969. Meristem-tip culture of chrysanthemums. Ann. Rept. Floric. Shodoshima Branch, Kagawa Agric. Exp. Sta. 1968: 1-12 (in Japanese).
22. Larsen, E. C. 1966. Daily temperature-cycles in heat inactivation of viruses in chrysanthemum and apple. Proc. Intern. Hort. Congr., 17th, College Park, Md. 1: 104 (after Nyland, G., and Goheen, A.C. 1969. Ann. Rev. Phytopath. 7: 331-354).
23. Miller, P. R. 1954. Detectin of virus B of chrysanthemum in the United States. FAO Plant Protection Bull. 2: 56-59.
24. Mori, K., Hamaya, E., Shimomura, T., and Ikegami, Y. 1969. Production of virus-free plants by means of meristem culture. J. Central Agric. Exp. Sta. 13: 45-110 (in Japanese).
25. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Planta. 15: 473-497.
26. Oertel, C. 1969. Zur partiellen Reinigung des B-Virus der Chrysantheme und Herstellung eines hochtiterigen Antiserum. Zentbl. Bakt. ParasitKde Abt. 2. 123: 277-281.
27. Stace-Smith, R., and Mellor, F. C. 1968. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. Phytopathology 58: 199-203.
28. Stone, O. M. 1963. Factors affecting the growth of carnation plants from shoot apices. Ann. appl. Biol. 52: 199-209.
29. Stone, O. M., and Hollings, M. 1969. Rept. Glasshouse Crops Res. Inst 1968: 109-110.
30. Tochihara, H. 1968. Three viruses isolated from chrysanthemum. Ann. Phytopath. Soc. Japan 34: 201 (Abstr.).
31. Tochihara, H. 1970. Some properties of chrysanthemum mild mottle virus, and comparison of this virus with cucumber mosaic virus. Ann. Phytopath. Soc. Japan 36: 1-10 (in Japanese).